201-14297

Jodi Burgess

02/12/2003 09:27 AM

To: Jodi Burgess/DC/USEPA/US@EPA, Sharon Coleman/DC/USEPA/US@EPA
cc: Mary-Beth Weaver/DC/USEPA/US@EPA
cc: Mary-Beth Weaver/DC/USEPA/US@EPA
Subject: Submission of Test Plan and Robust Summaries for HMPCC

----- Forwarded by Ralph Northrop/DC/USEPA/US on 02/11/2003 03:41 PM -----

"Adams, Tim" <tadams@therobertsgroup.net> on 12/03/2002 11:31:56 AM

To: "'Northrup.Ralph@epamail.epa.gov'" <Northrup.Ralph@epamail.epa.gov>
cc:
Subject: Submission of Test Plan and Robust Summaries for HMPCC

Dear Ms. Whitman: Attached are electronic files of the submission letter, test plan, and robust summaries for HMPCC submitted by the Alicycloc Aldehyde Consortium, a consortium in the Flavor and Fragrance High Production Volume Consortia (FFHPVC). Please confirm the receipt of these documents by return email. If you encounter any problems with the transmission of the documents, please feel free to contact me at any time.
Best regards,
Timothy Adams, Ph.D.
Technical Contact Person for FFHPVC

<<Robust Summaries for HMPCC.pdf>> <<Test Plan for HMPCC.pdf>>
<<Submission Letter for HMPCC.doc>>

- Robust Summaries for HMPCC.pdf
- Test Plan for HMPCC.pdf
- Submission Letter for HMPCC.doc

The Flavor and Fragrance High Production Volume Consortia
(FFHPVC)
1620 I Street, N.W.
Suite 925
Washington D.C. 20006
Tel. (202)-331-2325 Fax (202)-463-8998

December 3, 2002

Christie Todd Whitman, Administrator
US EPA
P.O. Box 1473
Merrifield, VA 22116
Attn: Chemical Right-to-Know Program

Dear Ms. Whitman:

On bchalf or the member companies of the Alicyclic Aldehyde Consortium, the Flavor and Fragrance High Production Volume Consortia is pleased to submit the Test Plan and Robust Summaries for the substance designated as "HMPCC" to the HPV Challenge Program, AR-201. The Alicyclic Aldehyde Consortium has chosen not to belong to the HPV Tracker System for submission of test plans and robust summaries. We are therefore submitting the test plan and accompanying robust summaries directly to EPA to make available to the public. This submission includes one electronic copy in pdf. format. A hard copy of this submission is available upon request. The EPA registration number for the Alicyclic Aldehyde Consortium is

Please feel free to contact me with any questions or comments you might have concerning the submission at tadams@therobertsgroup.net, tadams@chemintox.com or 202-331-2325.

Sincerely,
Timothy Adams, Ph.D.
Technical Contact Person for FFHPVC

201-14297A

The Flavor and Fragrance High Production Volume Consortia

The Alicyclic Aldehyde Consortium

Test Plan for HMPCC

3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (HMPCC) **CAS No. 31906-04-4**

FFHPVC Alicyclic Aldehyde Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

The Flavor and Fragrance High Production Volume Chemical Consortia

1620 I Street, NW, Suite 925

Washington, DC 20006

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List of Member Companies

INTERNATIONAL FLAVORS & FRAGRANCES INC.

TAKASAGO INTERNATIONAL CORP.

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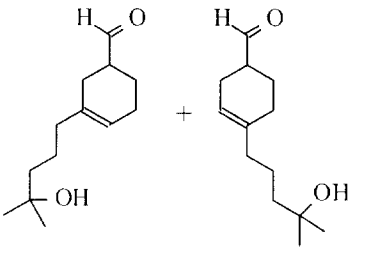
The Flavor and Fragrance High Production Volume Consortia

Test Plan for HMPCC

1 IDENTITY OF SUBSTANCE



3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde

CAS No. 31906-04-4

Synonyms:

HMPCC
3-Cyclohexen-1-carboxaldehyde, 4-(4-hydroxy-4-methylpentyl)-
Hydroxyisohexyl 3-cyclohexen carboxaldehyde
4-(4-Hydroxy-4-methylpentyl)cyclohex-3-enecarbaldehyde
Kovanol

2 CHEMICAL ANALYSIS

2.1 INTRODUCTION

In October of 1999, members of the U.S. flavor and fragrance industries as well as other manufacturers that produce source materials used in flavors and fragrances formed consortia of companies in order to participate in the Chemical Right-to-Know Program. Members of these consortia are committed to assuring the human and environmental safety of substances used in flavor and fragrance products. The consortia are organized as the Flavor and Fragrance High Production Volume Consortia (FFHPVC). The Alicyclic Aldehyde Consortium, as a member of FFHPVC, serves as an industry consortium to coordinate testing activities for alicyclic aldehyde substances under the Chemical Right-to-Know Program. Two (2) companies are current members of the Alicyclic Aldehyde Consortium. The Consortium and its member companies are committed to assembling and reviewing available test data, developing and providing test plans for each of the sponsored chemicals, and where needed, conducting additional testing. The test plan, category analysis and robust summaries presented represent the first phase of the Consortium's commitment to the Chemical Right-to-Know Program.

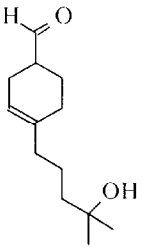
2.2 BACKGROUND INFORMATION

This category analysis and test plan provides data for 3- and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde (from herein referred to as HMPCC) and its structural relatives, hydroxycitronellal, hydroxycitronellol, and perilla aldehyde. HMPCC has not been reported to occur naturally. It is a colorless viscous liquid with a sweet aroma reminiscent of lily of the valley [Bauer and Garbe, 1985]. The functional aroma is similar to that of its naturally occurring counterpart, 7-hydoxycitronellal (*i.e.*, 7-hydroxy-3,7-dimethyloctanal). Therefore, it is not unexpected that HMPCC and 7-hydroxycitronellal contain the same functional groups (*i.e.*, an aldehyde and dimethyl substituted tertiary alcohol) distributed at either end of a carbon chain 8 to 10 carbons in length. Due to the method of preparation (see Section 2.4) HMPCC exists as

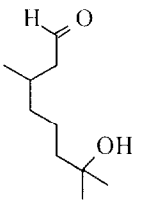
a 70:30 mixture of the 4- and 3-(4-hydroxy-4-methylpentenyl)-3-cyclohexenecarboxyaldehyde isomers. This mixture is the predominant product of commerce. At low concentrations, the mixture has excellent fixative properties, particularly in soap, cosmetics, and perfumes.

2.3 Structural Classification

The chemical structure of HMPCC and 7-hydroxycitronellal feature an aldehyde function and a dimethyl substituted tertiary alcohol located at either end of a carbon chain 8 to 10 carbons in length. In the case of HMPCC, the aldehyde is bonded to a cyclohexene ring that is substituted at the 4 position with a 4-hydroxy-4-methylpentyl substituent. The aldehyde group is separated from the dimethyl substituted tertiary alcohol moiety by seven carbons. In 7-hydroxycitronellal, the aldehyde is bonded to an eight carbon chain containing a 7-hydroxy-7-methyl substituent. In this case, the aldehyde group is separated from the dimethyl substituted tertiary alcohol by five carbons (see structure below).



HMPCC



7-Hydroxycitronellal

2.4 Industrial Production

HMPCC is synthesized by a Diels-Alder reaction in which the double bond of acrolein (2-propenal) adds to the 1- and 4-positions of myrcenol (6-methylene-2-methyl-7-octen-2-ol). At elevated temperature in the absence of a catalyst, the Diels-Alder orientation of addition of the acrolein unit to the diene of myrcenol yields a 30:70 mixture of 3- and 4-(4-hydroxy-4-methylpentyl)-3-cyclohexenecarboxaldehyde. The mixture is the typical product of commerce.

2.5 ABSORPTION, DISTRIBUTION, METABOLISM, AND EXCRETION

The structurally related substances 7-hydroxy-3,7-dimethyloctanal (*i.e.*, 7-hydroxycitronellal) and 4-isopropenyl-1-cyclohexenecarboxyaldehyde (*i.e.*, perilla aldehyde) participate in the same pathways of metabolism in rabbits, dogs, rats and humans. The predominant metabolic pathways involve either reduction of the aldehyde to yield the corresponding alcohol or oxidation of the aldehyde to yield the corresponding carboxylic acid. In both cases the metabolites are excreted as glucuronic acid conjugates predominantly in the urine.

Male rabbits (6) were administered an aqueous solution (20 ml) containing Tween 80 (0.02 g/100 ml) and 250-333 mg/kg bw of 7-hydroxycitronellal by stomach tube followed by 20 ml of water. Greater than 35% of the original dose is excreted in the urine as acidic and neutral metabolites within 72 hours [Ishida *et al.*, 1989]. The two principal urinary metabolites of 7-hydroxycitronellal are 7-hydroxycitronellic acid and 7-hydroxycitronellol isolated in a 5:2 ratio, respectively.

Another aldehyde structurally related to HMPCC, 4-isopropenyl-1-cyclohexenecarboxyaldehyde, commonly recognized as perilla aldehyde, was also investigated in the same study. Six male rabbits were given single oral doses of 666 to 800 mg/kg bw of 4-isopropenyl-1-cyclohexenecarboxyaldehyde by gavage followed by 20 ml of water. Approximately 50% of the original dose is isolated within 72 hours. The principal acidic urinary metabolites accounting for approximately 40% of the dose include corresponding carboxylic acid, 4-isopropenyl-1-cyclohexenecarboxylic acid and its aromatized derivative 4-isopropylbenzoic acid. The principal neutral metabolites accounting for approximately 10% of the dose include the corresponding alcohol, 4-isopropenyl-1-cyclohexenecarbinol and its dihydro isomer.

The group of substances, 4-isopropenyl-1-cyclohexenecarboxyaldehyde (perilla aldehyde), 4-isopropenyl-1-cyclohexenecarbinol (perillyl alcohol), and 4-isopropenyl-1-

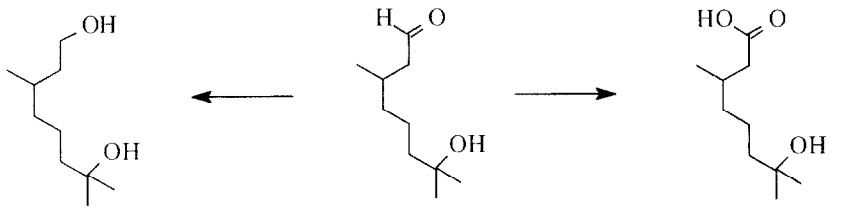
cyclohexenecarboxylic acid (perillic acid) have been the subject of extensive metabolic and toxicologic investigation in rats, dogs, and humans. The substances are currently being investigated as potential anti-carcinogenic agents for therapeutic use in humans. Patients with various advanced malignancies were treated with oral doses of 2,400 mg/m^2/dose of 4-isopropenyl-1-cyclohexenecarbinol for four weeks. Peak plasma levels for the two main metabolites occur at 1.5 hours (4-isopropenyl-1-cyclohexenecarboxylic acid) and 3.5 hours (4-isopropenylcyclohexanecarboxylic acid) post-ingestion. The parent alcohol is not detected in the plasma. The major acid metabolites as well as a small amount of 4-isopropenyl-1-cyclohexenecarbinol (less than 1%) are detected in the urine [Ripple *et al.*, 1998].

An *in vivo* study conducted in male Wistar rats, confirmed that the oxidation of 4-isopropenyl-1-cyclohexenecarbinol to 4-isopropenyl-1-cyclohexenecarboxylic acid involved 4-isopropenyl-1-cyclohexenecarboxaldehyde as an intermediate. Groups of rats were intravenously administered the perillyl alcohol, perilla aldehyde, or perillic acid at a dose of 80 micromoles/kg bw (approximately 12.2, 12.0, or 13.3 mg/kg bw, respectively). Urine and bile were collected for two consecutive hours post administration. In all cases, the glucuronic acid conjugate of 4-isopropenyl-1-cyclohexenecarboxylic acid was the predominant metabolite detected in the urine and bile. Based on the results, the authors concluded that within two hours, approximately 56% of the original dose is oxidized to 4-isopropenyl-1-cyclohexenecarboxaldehyde, followed by the conversion to 4-isopropenyl-1-cyclohexenecarboxylic acid, and eventually excretion as a glucuronic acid conjugate [Boon *et al.*, 2000].

Female Wistar-Furth rats fed a diet of 2% 4-isopropenyl-1-cyclohexenecarbinol for a period of 3, 5, or 10 weeks, show 4-isopropenyl-1-cyclohexenecarboxylic acid and 4-isopropenylcyclohexanecarboxylic acid as major plasma metabolites. Unchanged 4-isopropenyl-1-cyclohexenecarbinol is not detected. The same plasma metabolites are identified four hours after female Wistar-Furth rats are administered a single dose of 1,000 mg/kg 4-isopropenyl-1-cyclohexenecarbinol *via* gavage. No trace of 4-isopropenyl-1-cyclohexenecarbinol is found at any point, including 15 minutes post gavage. These results indicate that 4-isopropenyl-1-cyclohexenecarbinol is rapidly metabolized to 4-isopropenyl-1-

cyclohexenecarboxaldehyde and then to 4-isopropenyl-1-cyclohexenecarboxylic acid. [Haag and Gould, 1994]. Two beagle dogs (male and female) administered 250 mg/kg bw of 4-isopropenyl-1-cyclohexenecarbinol by gavage exhibit peak plasma levels of oxidized metabolites of 4-isopropenyl-1-cyclohexenecarbinol (*e.g.* 4-isopropenyl-1-cyclohexenecarboxylic acid and 4-isopropenylcyclohexanecarboxylic acid) at one and five hours post administration, respectively. Analysis of blood specimens collected before dosing and at 19 points ranging from 10 minutes to 48 hours after dosing, indicate the presence of the oxidized metabolites 10 minutes post administration. The parent substance, 4-isopropenyl-1-cyclohexenecarbinol, is undetectable in the plasma. [Phillips *et al.*, 1995].

Figure 1 - Metabolism of (±)-7-Hydroxy-3,7-dimethyloctanal in Rabbits



7-hydroxy-3,7-dimethyloctanol 7-hydroxy-3,7-dimethyloctanal 7-hydroxy-3,7-dimethyloctanoic acid

2.6 SUMMARY FOR CHEMICAL ANALYSIS

Based on pharmacokinetic and metabolic studies in rabbit, rats, dogs, and humans with 7-hydroxycitronellal and perilla aldehyde derivatives, it is anticipated that HMPCC will be rapidly absorbed *via* the oral route of exposure and primarily metabolized to the corresponding carboxylic acid and, to a lesser extent, the corresponding alcohol. Both metabolites are excreted primarily in the urine.

3 TEST PLAN

3.1 CHEMICAL AND PHYSICAL PROPERTIES

3.1.1 Melting Point

Being a mixture of 3- and 4-(4-hydroxy-4-methylpentenyl)-3-cyclohexene carboxyaldehyde, HMPCC is a viscous liquid at ambient temperature. The calculated melting point for a single isomer of HMPCC according to the MPBPWIN program is between 65.64 and 89.01 °C depending on the method used with a mean of 77.32 °C [MPBPVP EPI Suite, 2000]. Given that the commercial product is a mixture of the 3- and 4-(4-hydroxy-4-methylpentenyl)-3-cyclohexenecarboxyaldehyde, the determination of a melting point for the either the 3- or 4-isomer is not relevant. Based on these calculated values, the melting point of a single isomer of HMPCC is estimated to be 77.32 °C.

3.1.2 Boiling Point

The boiling point of HMPCC has been reported to be 280 °C [FMA, unpublished report] and 120-122 °C at 1.0 mm Hg [Bauer and Garbe, 1985]. The calculated boiling point for HMPCC according to the MPBPWIN program is 307 °C [MPBPVP EPI Suite, 2000]. This value is expected to be higher than that of 7-hydroxycitronellal, a structurally related substance also possessing the same two functional groups located at either end of the carbon skeleton, but containing three additional carbons. Therefore, the boiling point of HMPCC is anticipated to be higher than the 241 °C boiling point [FMA, unpublished report] (85-87 °C at 1.0 mm Hg [Bauer and Garbe, 1985]) reported for 7-hydroxycitronellal. Based on the consistency of the measured and calculated values, the boiling point of HMPCC is recognized to be 280 °C or 120-122 °C at a reduced pressure of 1.0 mm Hg.

3.1.3 Vapor Pressure

The calculated vapor pressure of HMPCC and 7-hydroxycitronellal has been reported to be 0.001 mm Hg at 20 °C [FMA, unpublished report]. The calculated vapor pressure for HMPCC according to the MPBPWIN program was 0.0000273 mm Hg at 25 °C [MPBPVP EPI Suite, 2000]. Although the vapor pressure of HMPCC determined from boiling point data is reported to be less than 0.001 mm Hg at 20 °C, it is recommended that the vapor pressure should be determined by a standardized methodology.

3.1.4 n-Octanol/Water Partition Coefficients

Log KOW was calculated resulting in values of 3.32 [KOWWIN EPI Suite, 2000] and 2.03 [Interactive Analysis LogP and LogW Predictor]. Based on calculated and measured values for 7-hydroxycitronellal, it is likely that the measured log KOW for HMPCC is less than the calculated value. The log KOW of 7-hydroxycitronellal was calculated to be 2.11 [KOWWIN EPI Suite, 2000] while the experimentally determined value is 1.5 [Procter and Gamble, 1996]. These conclusions should be validated by measurement of log KOW for HMPCC.

3.1.5 Water Solubility

The calculated water solubility was estimated to be 1,045 mg/L [Interactive Analysis LogP and LogW Predictor] and 184.6 mg/L at 25 °C [WSKOWIN EPI Suite, 2000]. Based on the limited data set, it is necessary to perform a water solubility test for HMPCC.

3.1.6 New Testing Required

- Vapor pressure for HMPCC according to an OECD Guideline protocol.
- Log KOW for HMPCC according to an OECD Guideline protocol.
- Water solubility for HMPCC according to an OECD Guideline protocol

3.2 ENVIRONMENTAL FATE AND PATHWAYS

3.2.1 Photodegradation

The calculated half-life value for HMPCC has been reported to be 1.009 hours [AOPWIN EPI Suite, 2000]. The short half-life is consistent with the presence of a reactive hydroxyl OH and an aldehyde function.

3.2.2 Stability In Water

HMPCC is expected to be stable in aqueous solution given that it contains an unreactive tertiary alcohol group and aldehyde that is not readily oxidizable in water.

3.2.3 Biodegradation

In a 20-day OECD closed bottle test, HMPCC showed measurable bio-oxidation (BOD/COD x100=10%) after 20 days incubation with activated sludge. Because HMPCC showed limited solubility in the test medium, it was directly injected into the reaction vessel. According to the authors, the test was intended to screen substances of potential biodegradation. [Waggy and Blessing, 1986]. The MITI linear and non-linear model predictions indicate that HMPCC should be readily biodegraded [BIOWIN EPI Suite, 2000].

The structurally related substance, 7-hydroxycitronellal at an initial dose of 52.5 mg DOC/l is completely biodegraded (99.8%) by day 19 in Method F biodegradability study in the Blue Book [Stickley, 1990]. In a more recent biodegradability test using a modified Sturm procedure in an OECD 301B Guideline, 7-hydroxycitronellal was 93.7% biodegraded after 28 days. According to the authors, hydroxycitronellal is classified as readily and ultimately biodegradable [King, 1994].

Although it is anticipated that HMPCC like 7-hydroxycitronellal will be readily and ultimately biodegradable, it is recommended that HMPCC be subjected to a biodegradability study according to a standard OECD Guideline protocol.

3.2.4 Fugacity

Transport and distribution in the environment were modeled using Level III Fugacity-based Environmental Equilibrium Partitioning Model [Mackay, 1991, 1996a, 1996b] through the EPA EPI Suite 2000 program. The input parameters used were molecular weight, melting point (77.3°C), vapor pressure (0.001 m Hg), and boiling point (280°C).

The model predicts that HMPCC is distributed mainly to the soil (74.5%), but also is distributed to water (24.7%) and, to some extent, air (0.015%) and sediment (0.81%).

3.2.5 New Testing Required

- Biodegradation study for HMPCC according to an OECD Guideline protocol.

3.3 ECOTOXICITY

3.3.1 Acute Toxicity to Fish

In a semi-static test with guppies, the structural relative 3-cyclohexene-1-carboxaldehyde exhibited a 14-day LC50 of 10.2 micromoles/L or 21.4 mg/L [Deneer *et al.*, 1988]. The calculated LC50 values for HMPCC are on the same order of magnitude. The calculated 96-hour LC50 for HMPCC was reported to be 6.8 mg/L (aldehydes) and the 14-day LC50 was reported to be 20. mg/L [ECOSAR EPI Suite, 2000].

Given the current database of information, it will be necessary to perform an acute fish toxicity test for HMPCC.

3.3.2 Acute Toxicity to Aquatic Invertebrates

Measured and calculated aquatic invertebrate LC50 values are available for HMPCC. Based on a protocol in EPA Methods for Toxicity Tests with Aquatic Organisms (40GTW23), the 48-hour LC50 for HMPCC in *Daphnia magna* was determined to be 76 mg/L [Waggy and Blessing, 1986]. In *Daphnia magna,* a calculated 48-hour LC50 of 1.773 mg/L was determined [ECOSAR EPI Suite, 2000].

Given the current database of information, it will not be necessary to perform additional acute aquatic invertebrate toxicity tests.

3.3.3 Acute Toxicity to Aquatic Plants

A calculated 96-hour EC50 of 7.091 mg/L was reported for green algae [ECOSAR EPI Suite, 2000].

No experimental data on the aquatic plant toxicity of HMPCC or a structurally related substance are available. Therefore, it is recommended that HMPCC be subjected to an acute toxicity test in green algae.

3.3.4 New Testing Required

- Acute toxicity test of HMPCC in fish according to OECD Guideline 203 protocol.
- Acute toxicity test of HMPCC in algae according to OECD Guideline 201 protocol.

3.4 HUMAN HEALTH TOXICITY

3.4.1 Acute Toxicity

The acute toxicity of HMPCC was reported to be low, with oral LC50s of 3,000 to greater than 5,000 mg/kg bw in rats and dermal LC50s of greater than 5000 mg/kg bw in rabbits [Opdyke, 1977; Mallory *et al.*, 1982; Myers *et al.*, 1987]. Similar results were reported with hydroxycitronellal [Opdyke, 1973].

Exposure to statistically generated saturated vapor of HMPCC for 6 hours, resulted in no deaths, no toxicity or no remarkable gross pathological lesions in exposed male or female rats [Myers *et al.*, 1987].

Groups of rats were exposed in a dynamic system to up to 558 ppm of 4,4-dimethyl-3-cyclohexenecarboxaldehyde vapor for 4 hours and observed for 14 days [Union Carbide, 1987]. Similarly groups of rats were exposed to up to 402 ppm 4,4-dimethyl-3-cyclohexenecarboxaldehyde vapor for 1 hour in a static system and observed for 14 days. Signs exhibited included lacrimation, peri-oral wetness and respiratory difficulties on day of exposure. No clinical signs or macroscopic lesions were reported post exposure. Some deaths occurred on days 1 or 2 post exposure at 558 ppm; however the authors considered the deaths to be related to exposure to acrolein, a reaction precursor and contaminant, since the 1-hour rat LC50 of acrolein is 26 ppm. Therefore, no mortalities were attributed 4,4-dimethyl-3-cyclohexenecarboxaldehyde exposure.

Given the results of oral, dermal, and inhalation studies, no additional acute toxicity tests in mammals are recommended.

3.4.2 *In vitro* and *In vivo* Genotoxicity

3.4.2.1 In vitro

HMPCC did not increase the number of revertants in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 or in *Escherichia coli* strain WP2 *uvr*A when tested with or without metabolic activation at concentrations up to 5,000 micrograms/plate [Wagner and Klug, 1999]. Another structural relative of HMPCC, 2,4-dimethyl-3-cyclohexene-1-carboxaldehyde, also did not increase the number of revertants in *Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538 when tested with or without metabolic activation [Vergnes and Morabit, 1995].

In Chinese hamster ovary cells, HMPCC (at concentrations tested up to 900 micrograms/ml) did not induce an increase in the number of chromosome aberrations in the presence or absence of S9 at any test concentration compared to control solutions [Gudi and Schadly, 2000].

3.4.2.2 In vivo

Groups of mice were intraperitoneally injected with up to 900 mg/kg bw of HMPCC in corn oil [Gudi and Krsmanovic, 2000]. At 24 or 48 hours, mice were killed, femurs were exposed, and bone marrow was removed. The number of micronucleated normochromatic erythrocytes was counted and the proportion of polychromatic erythrocytes to total erythrocytes was determined. HMPCC does not induce micronucleated polychromatic erythrocytes in the mouse bone marrow assay.

When administered to mice by intraperitoneal injection, 7-hydroxycitronellol (up to 1,204 mg/kg bw) and 7-ydroxycitronellal (up to 861 mg/kg bw) did not induce an increase in the incidence of micronuclei in mouse bone marrow [Wild *et al.*, 1983].

In *Drosophila*, 7-hydroxycitronellal (37 mM) or 7-hydroxycitronellol (10 mM) did not increase the number of sex-linked recessive lethal mutations as compared to controls [Wild *et al.*, 1983].

3.4.2.3 Conclusions

The genotoxicity database on HMPCC and 7-hydroxycitronellal shows no mutagenic potential in the Ames assay. In a mammalian assay, there was no evidence of an increase in the incidence of chromosomal aberrations in the presence or absence of S9. In whole animals, the genotoxicity results for HMPCC, 7-hydroxycitronellol, and 7-hydroxycitronellal showed no evidence of an induction of bone marrow micronuclei in mice. In *Drosophila*, 7-hydroxycitronellol or 7-hydroxycitronellal did not induce an increase in the number of sex-linked recessive lethal mutations. Based on these results no additional genotoxicity tests are recommended.

3.4.3 Repeat Dose Toxicity

Repeat-dose inhalation and oral studies have been conducted for structural relatives of HMPCC, including 7-hydroxycitronellal, dimethyl-3-cyclohexenecarboxaldehyde, and perilla aldehyde (4-isopropenyl-1-cyclohexenecarboxyaldehyde) derivatives.

3.4.3.1 Subacute Studies

Rats were exposed to 0, 50, 125 or 250 ppm of 4,4-dimethyl-3-cyclohexenecarboxaldehyde vapor, 6 hours/day for 9 exposures [Norris and Kintigh, 1994]. Additional rats were assigned to the control and high concentration groups for inclusion in a 4-week recovery period. No overt signs of toxicity were reported. There was an increased incidence of higher values for bilirubin, urobilinogen and amorphous phosphates in all treated males on day 11 and an increased incidence of bilirubin and urobilinogen in females at the highest concentration on day 12. Other reported effects included initial decreased body weight gain, increased water consumption, increased serum urea nitrogen values, exposure-related increase in renal tubular

immunohistochemical staining for *alpha*-2micro-globulin in males, increased relative liver (mid- and high doses) and kidney weights (high dose) in males, swollen periocular tissue (mid- and high doses), periocular encrustation (high dose), alopecia (high dose) corneal lesions (high-dose), and increased urine osmolalities in males on day 11 (mid- and high doses). After the recovery period, females exposed to the highest concentration had decreased total erythrocytes, hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration values, showed increased segmented neutrophils and decreased monocytes, and showed slightly increased protein in the urine. The authors considered that dimethyl-3-cyclohexenecarboxaldehyde appears to be an ocular and respiratory irritant at vapor concentrations of 125 ppm and higher. There were no observable adverse effects reported at 50 ppm.

3.4.3.1 Subchronic Studies

Groups of female rats or Syrian golden hamsters were exposed to 211 micrograms of 7-hydroxycitronellal/cubic meters as part of a complex fragrance mixture (50 mg/cubic meters for 4 hours/day, 5 days/week for 13 weeks [Fukayama *et al.*, 1999]. Twelve animals per test group were exposed in a whole body inhalation experiment. Aerodynamic mean diameter of particle size was 0.5 um in rats and 1.4 um in hamsters. Animals were sacrificed 1 to 2 days following exposure. Hematological examination at week 13, involved measurement of white blood cell count, mean corpuscular volume, hemoglobin concentration, and hematocrit. Clinical chemistry examinations were performed at weeks 6 or 7 and week 13. At necropsy, gross pathological examination was performed on 24 organs and tissues including the uterus, testes and ovaries. Histopathological examination was performed on the trachea, lungs, adrenals, brain, esophagus, heart, kidneys, liver pancreas, spleen, sternum, testes, uterus and bone marrow taken from the femur. No toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters were reported and no gross pathological or histopathological findings were observed in either species.

Numerous animal studies have shown high dietary levels of perilla aldehyde (4-isopropenyl-1-cyclohexenecarboxyaldehyde), its corresponding alcohol and acid may be protective against known animal carcinogens. The perilla aldehyde metabolite, 4-isopropenyl-1-cyclohexenecarbinol (discussed above in Section 2.5), has been shown to inhibit the growth of pancreatic, mammary, and liver tumors in animals. It has been studied in animals as a chemotherapeutic agent for neuroblastoma, prostate and colon cancer, and has possible chemotherapeutic applications for skin and lung cancer (Belanger, 1998; Crowell, 1997; Stark *et al.*, 1995; Burke *et al.*, 1997; Mills *et al.*, 1995; Ren and Gould, 1998; Haag and Gould, 1994; Reddy *et al.*, 1997: no robust summaries included).

In a 90-day study, dose levels 40, 120, or 400 mg/kg bw per day of 4-isopropenyl-1-cyclohexenecarbinol was given by gavage to groups of rats and dogs of unspecified number and strain [National Cancer Institute, 1996]. Clinical signs observed with increasing dose included hyper-excitement and a clear mouth discharge. The animals were provided food and drinking water *ad libitum* and monitored for survival, behavior and changes in hematology. In rats, no agent related deaths, abnormal hematology, clinical chemistry or gross lesions were reported at dose levels up to and including 400 mg/kg bw per day. Histopathological examination of major organs and tissues including the ovaries and gonads failed to reveal any alterations that could be associated with administration of the test substance [National Cancer Institute, 1996].

3.4.3.2 Chronic Studies

Two groups of male and female rats were fed 7-hydroxycitronellal at dietary concentrations of 0.1% (10 rats/sex) or 0.5% (20 rats/sex), respectively, for 2 years. These levels correspond to calculated average daily intakes of 50 or 250 mg 7-hydroxycitronellal/kg bw. Control animals were fed a basal diet. Animals were observed for appearance and behavior and body weights were determined regularly during the study. At the end of the study, rats were necropsied and microscopic examinations were performed on the liver, heart, pancreas, adrenals, spleen, brain, and gross lesions. The number of animals that survived the 2-year duration of the study was 5of 10 and 31 of 40, respectively. Low survival was attributed to the occurrence of spontaneous

diseases that occurred at the same rate in both test and control animals. Administration of 7-hydroxycitronellal at dose levels up to 250 mg/kg bw/d resulted in no evidence of systemic toxicty [Bar and Griepentrog, 1967].

3.4.4 Reproductive Toxicity

No reproductive toxicity study on HMPCC or a related compound was available.

3.4.5 Teratogenicity/Developmental Toxicity

No teratogenicity or developmental toxicity study on HMPCC or a related substance was available.

3.4.6 New Testing Required

- A reproductive/developmental screening assay of HMPCC according to OECD 421 Guideline protocol.

3.5 TEST PLAN TABLE

Chemical	Physical-Chemical Properties				
	Melting Point	Boiling Point	Vapor Pressure	Partition Coefficient	Water Solubility
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	Calc	A, Calc	Test, Calc	Test, Calc	Test, Calc

Chemical	Environmental Fate and Pathways			
	Photodegradation	Stability in Water	Biodegradation	Fugacity
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	Calc	NA	Test, A, R, Calc	Calc

Chemical	Ecotoxicity		
	Acute Toxicity to Fish	Acute Toxicity to Aquatic Invertebrates	Acute Toxicity to Aquatic Plants
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	Test, Calc	A, Calc	Test, Calc

Chemical	Human Health Data					
	Acute Toxicity	Genetic Toxicity *In Vitro*	Genetic Toxicity *In Vivo*	Repeat Dose Toxicity	Repro-ductive Toxicity	Develop-mental Toxicity
CAS No. 31906-04-4 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde	A	A	A	R	Test	Test

Legend	
Symbol	**Description**
R	Endpoint requirement fulfilled using category approach, SAR
Test	Endpoint requirements to be fulfilled with testing
Calc	Endpoint requirement fulfilled based on calculated data
A	Endpoint requirement fulfilled with adequate existing data
NR	Not required per the OECD SIDS guidance
NA	Not applicable due to physical/chemical properties
O	Other

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201-14297B

The Flavor And Fragrance High Production Volume Consortia

The Alicyclic Aldehyde Consortium

Robust Summaries for HMPCC

3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde

CAS No. 31906-04-4

FFHPVC Alicyclic Aldehyde Consortium Registration Number

Submitted to the EPA under the HPV Challenge Program by:

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TAKASAGO INTERNATIONAL CORP.

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The Flavor and Fragrance High Production Volume Consortia

Robust Summaries for HMPCC

The evaluation of the quality of the following data uses a systematic approach described by Klimisch [Klimisch *et al.*, 1996]. Based on criteria relating to international testing standards for categorizing data reliability, four reliability categories have been established. The following categories are:

- Reliability code 1. Reliable without restrictions
- Reliability code 2. Reliable with restrictions
- Reliability code 3. Not reliable
- Reliability code 4. Not assignable

1 CHEMICAL AND PHYSICAL PROPERTIES

1.1 Melting Point

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Calculated/Adapted Joback Method
GLP	No
Melting Point	89.01 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Calculated/Gold and Ogle Method
GLP	No
Melting Point	65.64 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Calculated/Mean of Joback and Gold and Ogle Methods
GLP	No
Melting Point	77.32 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

1.2 Boiling Point

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Measured
GLP	No

Year	1985
Boiling Point	120 - 122 °C
Pressure	1.0 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Bauer K. and Garbe D. (1985) Common Flavor and Fragrance Materials Verlagsgesellschaft mbH, D-6940, Weinheim, Federal Republic of Germany.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Substance	Data is for structurally related substance 7-hydroxycitronellal
Method/guideline	Measured
GLP	No
Year	1987
Boiling Point	85 - 87 °C
Pressure	1.0 mm Hg
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Bauer K. and Garbe D. (1985) Common Flavor and Fragrance Materials Verlagsgesellschaft mbH, D-6940, Weinheim, Federal Republic of Germany.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Calculated/Adapted Stein and Brown method
GLP	No
Boiling Point	307.07 °C
Pressure Unit	mm Hg
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.

References	MPBPVP EPI Suite (2000) v1.40 U S Environmental Protection Agency.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Calculated
Boiling Point	280 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Fragrance Materials Association (FMA) Unpublished report to RIFM.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Substance	Data is for structurally related substance 7-hydroxycitronellal
Method/guideline	Calculated
Boiling Point	241 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4.Only secondary literature (review, tables, books, etc.).
References	Fragrance Materials Association (FMA) Unpublished report to RIFM.

1.3 Vapor Pressure

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Calculated
GLP	No

Vapor Pressure	Less than 0.001 mm Hg
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Unpublished report to RIFM.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Calculated/Modified Grain method
GLP	No
Vapor Pressure	0.0000273 mm Hg
Temperature	25 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	MPBPVP EPI Suite (2000) U S Environmental Protection Agency.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Substance	Data is for structurally related substance 7-hydroxycitronellal
Method/guideline	Calculated
GLP	No
Vapor Pressure	Less than 0.001 mm Hg
Temperature	20 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Fragrance Materials Association (FMA) Unpublished report to RIFM.

1.4 n-Octanol/Water Partition Coefficients

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Substance	Data is for structurally related substance 7-hydroxycitronellal
Method/guideline	Measured
GLP	Ambiguous
Year	1996
Log Pow	1.5
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Acceptable, well-documented publication/study report, which meets basic scientific principles.
References	Procter and Gamble Company (1996) Unpublished submission to EPA.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Calculated
GLP	No
Log Pow	2.03
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Interactive Analysis LogP and LogW Predictor: Database contributed by Syracuse Research Corporation, SciVision, Albany Molecular Research, Inc., eduSoft LC, Cambridge Soft. www.logp.com.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Substance	Data is for structurally related substance 7-hydroxycitronellal
Method/guideline	Calculated
GLP	No

Log Pow	2.11
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	KOWWIN EPI Suite (2000) U S Environmental Protection Agency.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Calculated
GLP	No
Log Pow	3.32
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	KOWWIN EPI Suite (2000) U.S. Environmental Protection Agency.

1.5 Water Solubility

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/Guideline	Calculated from Log Kow
GLP	No
Value (mg/L) at Temperature	184.6 mg/L at 25 °C
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	WSKOW EPI Suite (2000) U S Environmental Protection Agency.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
GLP	No
Value (mg/L) at Temperature	1,045 mg/L
Remarks for Test Conditions	No temperature given.
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Interactive Analysis LogP and LogW Predictor: Database contributed by Syracuse Research Corporation, SciVision, Albany Molecular Research, Inc., eduSoft LC, Cambridge Soft. www.logp.com.

2 ENVIRONMENTAL FATE AND PATHWAYS

2.1 Photodegradation

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Calculated
Test Type	AOPWIN
Halflife t1/2	1.009 hours
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	AOPWIN EPI Suite (2000) U S Environmental Protection Agency.

2.2 Biodegradation

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method	"Standard Methods", authors did not provide detail
Test Type	Measured
GLP	Ambiguous
Year	1985
Remarks for Test Conditions	Test substance added by injection directly into BOD bottle due to limited solubility.
Degradation % After Time	Bio-oxidation (BOD/CODx100) = 10% on day 20
Results	Measured theoretical oxygen demand in mg O2/mg compound = 3.13

Time required for 10% degradation	20 days
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
Reference	Waggy G.T. and Blessing, R.L. [1986] Ecological fate and effects testing of UCC products and wastewaters during 1985. UCC Business Confidential, Project Report dated March 11, 1986. Central Engineering Department, Union Carbide Corporation.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Substance	Data is for structurally related substance 4,4-dimethyl-3-cyclohexenecarboxaldehyde
Method	"Standard Methods", authors did not provide detail
Test Type	Measured
GLP	Ambiguous
Year	1985
Remarks for Test Conditions	Test substance added by injection directly into BOD bottle due to limited solubility
Degradation % After Time	Biooxidation (BOD/CODx100) = 10% on day 20
Results	Measured theoretical oxygen demand in mg O2/mg compound = 2.75
Time required for 10% degradation	20 days
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
Reference	Waggy G.T. and Blessing R.L. [1986] Ecological fate and effects testing of UCC products and wastewaters during 1985. UCC Business Confidential, Project Report dated March 11, 1986. Central Engineering Department, Union Carbide Corporation.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Substance	Data is for structurally related substance 7-hydroxycitronellal
Method	Method F from the Blue Book Series, Assessment of Biodegradability 1981

	Biodegradability 1981
Test Type	Measured
GLP	No
Year	1990
Contact time (units)	28 days
Innoculum	Activated sludge from sewage
Remarks for Test Conditions	The test material was diluted to 52.5 mg DOC/L in buffered solution containing 30 mg activated sludge solids/L. The mixture was agitated at 20 °C over 28 days with periodic measurements of dissolved organic carbon.
Degradation % After Time	As % removal of DOC: day 0, 1, 2, 5, 7, 9, 12, 15, and 19 was 0, 6.2, 0.8, 46, 84.1, 90.5, 91.9, 97.9, and 99.8, respectively.
Results	A better than 95% COC removal was achieved within 19 days and therefore the study was terminated.
Time required for 10% degradation	Less than 5 days
10 day window criteria	Yes
Total degradation	99.8% at 19 days
Conclusion Remarks	Hydroxycitronellal has a high degree of biodegradability.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
Reference	Stickley D.P. (1990) Report to Bush Boake Allen Limited on Biodegradability of Citral 900UC and Hydroxy Citronellal Pure 55. Berridge Environmental Laboratories Limited. Report No. 8347 dated February 15, 1990.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Substance	Data is for structurally related substance 7-hydroxycitronellal (purity 95.0%)
Method	Sealed vessel test (OECD Guideline 301B)
Test Type	CO2 production test
GLP	Ambiguous
Year	1990
Contact time (units)	28 days

Innoculum	Secondary effluent from an unacclimatized activated sludge plant
Remarks for Test Conditions	The test concentration was nominal 10 mg/L organic carbon with a test temperature range of 20 to 24 °C. Sealed vessels were incubated on a rotary shaker.
Degradation % After Time	For day 4, 7, 11, 14, 18, 21, 25, and 28 was 3.8, 64.7, 66.3, 78.3, 80.5, 90.5, 85.5, and 93.7, respectively.
Results	95% confidence interval of 87.9 to 99.5
Time required for 10% degradation	5 days
Classification	Readily and ultimately biodegradable.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
Reference	King J.M.H. (1994) The Biodegradability of Perfume Ingredients. Unilever Research.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method	Linear model prediction; non-linear model prediction; MITI linear model prediction; MITI non-linear model prediction
Test Type	Calculated
GLP	No
Results	Biodegrades fast.
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	BIOWIN EPI Suite (2000) U S Environmental Protection Agency.

2.3 Fugacity

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Model Conditions	25 C, 100,000 lbs
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III Fugacity-based Environmental Equilibrium Partitioning Model
Remarks for Test Conditions	The input parameters used were molecular weight, melting point (77.32 °C) and boiling point (307.07 °C).
Input Parameters	MW, calculated VP, calculated MP
Media	Air
Estimated Distribution and Media Concentration	0.0153%
Model data and results	half life = 0.486 hrs for 1000 kg/hr emission rate
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five stage process. *Environmental Toxicology and Chemistry*, **15(9),** 1618-1626. Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. *Environmental Toxicology and Chemistry*, **15(9),** 1627-1637.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Model Conditions	25 C, 100,000 lbs
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III Fugacity-based Environmental Equilibrium Partitioning Model

	Model
Remarks for Test Conditions	The input parameters used were molecular weight, melting point (77.32 °C) and boiling point (307.07 °C).
Input Parameters	MW, calculated VP, calculated MP
Media	Water
Estimated Distribution and Media Concentration	25.5%
Model data and results	half life=900 hrs for 1000 kg/hr emission rate
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five stage process. *Environmental Toxicology and Chemistry*, **15(9),** 1618-1626. Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. *Environmental Toxicology and Chemistry*, **15(9),** 1627-1637.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Model Conditions	25 C, 100,000 lbs
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III Fugacity-based Environmental Equilibrium Partitioning Model
Remarks for Test Conditions	The input parameters used were molecular weight, melting point (77.32 °C) and boiling point (307.07 °C).
Input Parameters	MW, calculated VP, calculated MP
Media	Soil
Estimated Distribution and Media Concentration	74.5%
Model data and results	half life = 900 hrs for 1000 kg/hr emission rate
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a

five stage process. *Environmental Toxicology and Chemistry*, **15(9),** 1618-1626.

Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. *Environmental Toxicology and Chemistry*, **15(9),** 1627-1637.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Model Conditions	25 C, 100,000 lbs
Test Type	Environmental Equilibrium Partitioning Model
Method	Mackay
Model Used	Level III Fugacity-based Environmental Equilibrium Partitioning Model
Remarks for Test Conditions	The input parameters used were molecular weight, melting point (77.32 °C) and boiling point (307.07 °C).
Input Parameters	MW, calculated VP, calculated MP
Media	Sediment
Estimated Distribution and Media Concentration	0.81%
Model data and results	half life= 3600 hrs for 0 kg/hr emission rate
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
References	Mackay D., A.DiGuardo, S.Paterson, G.Kicsi and C.E.Cowan (1996a) Assessing the fate of new and existing chemicals: a five stage process. *Environmental Toxicology and Chemistry*, 15(9), 1618-1626. Mackay D., A.DiGuardo, S.Paterson and C.E.Cowan (1996b) Evaluating the fate of a variety of types of chemicals using the EQC model. Environmental Toxicology and Chemistry, 15(9), 1627-1637.

3 ECOTOXICITY

3.1 Acute Toxicity to Fish

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Substance	Data is for structurally related substance 3-cyclohexene-1-carboxaldehyde
Method/guideline	Acute lethal toxicity (14-day LC50) - semi static
Test Type	Experimental
GLP	No
Year	1988
Species/Strain/Supplier	Guppy (*Poecilia reticulata*)
Exposure Period	14 days
Analytical monitoring	GC Analysis
Remarks for Test Conditions	Over 14 days, groups of 10 guppies (2-3 months old) were exposed to five concentrations of test substance in 1.5 L glass vessels containing 1.41 L test solution which was renewed daily. Control fish were exposed to 72 ul acetone/L. O2 content, pH and test substance concentration were determined before and after test solution renewal. LC50s were determined using logit transformation and corrected for loss of test substance.
Unit	umol/L
Endpoint value	LC50 = 10.2 (21.4 mg/L)
Remarks fields for results	LC50 reported at log 1.01. The LC50 was not corrected for loss of test substance because no reliable recovery factors could be determined. The authors attributed this to irreproducible results during analysis of the aqueous solution. pH values ranged from 6.5-7.5. O2 content was often low (3 mg/L) 24 hours following preparation of test solutions. The authors attributed this to bacterial growth and disregarded data from tests with low O2 content accompanied by mortality.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
Reference	Deneer J.W., Seinen, W., Hermens, J.L.M. (1988) The acute toxicity of aldehydes to the guppy. Aquatic Toxicol 12:185-192.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	ECOSAR
Test Type	Calculated
GLP	No
Species/Strain/Supplier	Fish
Exposure Period	96 hour
Remarks for Test Conditions	Based on: log KOW = 3.32, MP = 77.32 °C, water solubility = 49.71 mg/L
Unit	mg/L
Endpoint value	LC50 = 6.787
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	ECOSAR
Test Type	Calculated
GLP	No
Species/Strain/Supplier	Fish
Exposure Period	14 days
Remarks for Test Conditions	Based on: log KOW = 3.32, MP = 77.32 °C, water solubility = 49.71 mg/L
Unit	mg/L
Endpoint value	LC50 = 20.006
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

3.2 Acute Toxicity to Aquatic Invertebrates

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	EPA Committee on Methods for Toxicity Tests with Aquatic Organisms
Test Type	Experimental
GLP	Ambiguous
Year	1985
Analytical procedures	Not stated
Species/Strain/Supplier	*Daphnia magna*
Test Details	48 hours
Remarks for Test Conditions	Groups of 10 very young (less than 2 days) *Daphnia magna* were exposed to 5-10 concentrations of test substance or control in 250 ml beakers containing 200 ml test solution over a period of 48 hours. Dissolved oxygen and pH were determined at the beginning of the test and after 48 hours. Mortalities were recorded at 24 and 48 hours. Kanawha River water was used in the test and analyses were conducted: total hardness = 55 mg/L as CaCO3 total alkalinity = 36 mg/L as CaCO3 pH= 6.7 conductivity = 250 umhos/cm
EC50, EL50, LC0, at 24,48 hours	48-hour LC50 = 76 mg/L
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Data Reliability Remarks	Code 2. Basic data given: comparable to guidelines/standards.
Reference	Waggy G.T., Blessing, R.L. (1986) Ecological fate and effects testing of UCC products and wastewaters during 1985. UCC Business Confidential, Project Report dated March 11, 1986. Central Engineering Department, Union Carbide Corporation.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Substance	Data is for structurally related substance 4,4-dimethyl-3-cyclohexenecarboxaldehyde

	cyclohexenecarboxaldehyde
Method/guideline	EPA Committee on Methods for Toxicity Tests with Aquatic Organisms
Test Type	Experimental
GLP	Ambiguous
Year	1985
Analytical procedures	Not stated
Species/Strain/Supplier	*Daphnia magna*
Test Details	48 hours
Remarks for Test Conditions	Groups of 10 very young (less than 2 days) *Daphnia magna* were exposed to 5-10 concentrations of test substance or control in 250 ml beakers containing 200 ml test solution over a period of 48 hours. Dissolved oxygen and pH were determined at the beginning of the test and after 48 hours. Mortalities were recorded at 24 and 48 hours. Kanawha River water was used in the test and analyses were conducted: total hardness = 55 mg/L as CaCO3 total alkalinity = 36 mg/L as CaCO3 pH = 6.7 conductivity = 250 umhos/cm
EC50, EL50, LC0, at 24,48 hours	48-hour LC50 = 76 mg/L
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Data Reliability Remarks	Code 2. Basic data given: comparable to guidelines/standards.
Reference	Waggy G.T., Blessing, R.L. (1986) Ecological fate and effects testing of UCC products and wastewaters during 1985. UCC Business Confidential, Project Report dated March 11, 1986. Central Engineering Department, Union Carbide Corporation.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Calculated
Test Type	ECOSAR
Species/Strain/Supplier	Daphnia magna
Test Details	48 hours
EC50, EL50, LC0, at 24,48 hours	LC50 = 1.733 mg/L

Data Qualities Reliabilities	Reliability code 4. Not assignable.
Data Reliability Remarks	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U S Environmental Protection Agency.

3.3 Acute Toxicity to Aquatic Plants

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	ECOSAR
Test Type	Calculated
GLP	No
Species/Strain/Supplier	Green algae
Exposure Period	96 hours
Remarks for Test Conditions	Based on: log KOW = 3.32, MP = 77.32 °C, water solubility = 49.71 mg/L
Endpoint Value	EC50 = 7.091 mg/L
Data Qualities Reliabilities	Reliability code 4. Not assignable.
Remarks for Data Reliability	Code 4. Calculated.
Reference	ECOSAR EPI Suite (2000) U.S. Environmental Protection Agency.

4 HUMAN HEALTH TOXICITY

4.1 Acute Toxicity

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Test Type	Acute oral toxicity
GLP	No
Year	1977
Species/strain	Rat
Sex	Not reported
# of animals per sex per dose	10
Route of Administration	Oral
Remarks for Test Conditions	Rats were orally administered HMPCC, 5000 mg /kg bw and observed for 14 days.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Number of deaths at each dose level	2 deaths on day 1
Remarks for Results	Rats exhibited slight lethargy, tremors, flaccid tone and piloerection. At necropsy, 2 rats were reported to have dark livers, 2 rats had light yellow intestines and 1 rat had dark kidney.
Conclusion remarks	The oral LD50 of HMPCC in rats was reported to be greater than 5000 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Opdyke D.L. (1977) Acute Oral Toxicity in Rats. Dermal Toxicity in Rabbits, Lyral. Unpublished report to RIFM.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Test Type	Acute oral toxicity

GLP	Yes
Year	1982
Species/strain	Rat/Sprague-Dawley
Sex	Male and Female
# of animals per sex per dose	5
Route of Administration	Oral-Gavage
Remarks for Test Conditions	Groups of 5 rats/sex were administered a single dose of HMPCC, 4.0, 4.5, 5.0, 5.5 or 6.0 ml/kg bw by gavage and observed for 14 days.
Value LD50 or LC50 with confidence limits	Greater than 5,000 ml/kg bw
Number of deaths at each dose level	4.0 ml/kg bw: 2/5 males, 1/5 females 4.5 ml/kg bw: 0/5 males, 3/5 females 5.0 ml/kg bw: 1/5 males, 3/5 females 5.5 ml/kg bw: 1/5 males, 3/5 females 6.0 ml/kg bw: 2/5 males, 3/5 females
Remarks for Results	Necropsy findings of rats dying on the study included distended fluid-filled intestines and bladder, bright red lungs and blanched adrenals. Necropsy of surviving animals showed no remarkable findings.
Conclusion remarks	HMPCC shows a low order of acute oral toxicity in rats with an LD50 greater than 5000 ml/kg bw.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Mallory V.T., Naismith, R.W., Matthews, R.J. (1982) Acute oral toxicity study in rats (14 day). PH 402-IFF-005-81. 81-218-01. Pharmakon Research International Inc., Waverly, PA.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data is for structurally related substance 7-hydroxycitronellal
Test Type	Acute oral toxicity
GLP	No
Year	1973
Species/strain	Rat

Sex	Not reported
# of animals per sex per dose	10
Route of Administration	Oral
Remarks for Test Conditions	Rats were orally administered 7-hydroxycitronellal, 5000 mg/kg and observed for 14 days.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg
Number of deaths at each dose level	1/10 on day 7 1/10 on day 11
Remarks for Results	No symptomatology reported.
Conclusion remarks	The oral LD50 of 7-hydroxycitronellal in rats was reported to be greater than 5,000 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Opdyke D.L. (1973) Acute oral toxicity in rats. Dermal toxicity in rabbits. Unpublished report to RIFM.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Purity greater than 88%; 0.02% acrolein; less than 10% myrac, less than 2.5% myrcenol; mol wt = 210.3
Test Type	Acute oral LD50
GLP	No
Year	1987
Species/strain	Rat
Sex	Male and Female
# of animals per sex per dose	5
Route of Administration	Oral-Gavage
Remarks for Test Conditions	Groups of male and female rats were administered a single dose of HMPCC, 2.0, 4.0, 8.0 or 16.0 ml/kg bw by perioral intubation and observed for 14 days. Five female rats also received 1.0 ml/kg bw of HMPCC.
Value LD50 or LC50 with confidence limits	LD50 for males = 7.46 ml/kg bw (95% CL = 5.16-10.8) LD50 for females = 3.25 ml/kg bw (95% CL = 2.02-5.24)
Number of deaths at each dose level	Deaths occurred between 4 hours and 3 days following treatment.

dose level	treatment. 1.0 ml/kg bw - 0/5 females 2.0 ml/kg bw - 0/5 males; 1/5 females 4.0 ml/kg bw - 0/5 males; 3/5 females 8.0 ml/kg bw - 3/5 males; 5/5 females 16 ml/kg bw - 5/5 males; 2/2 females
Remarks for Results	Signs exhibited included sluggishness, lacrimation, tremors, kyphosis, red discharge around mouth, nose and eyes, unkempt appearance and prostration. Necropsy of affected rats showed pink or red lungs, distended and gas-filled stomachs and some gas-filled intestines. Survivors showed recovery within 1-5 days and had no remarkable lesions.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Myers R.C., Slesinski R.S., Frank F.R. (1987) Lyral (crude) [4-(4-hydroxy-4-methyl pentyl)-3-cyclohexene-1-carboxaldehyde]. Acute Toxicity and Primary Irritancy Studies. Bushy Run Research Center, Export, PA. Report No. 49-180 dated February 6, 1987.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Test Type	Acute dermal toxicity
GLP	No
Year	1977
Species/strain	Rabbit
Sex	Not reported
# of animals per sex per dose	10
Route of Administration	Dermal
Remarks for Test Conditions	Rabbits were dermally treated with HMPCC, 5000 mg/kg bw and observed for 14 days.
Value LD50 or LC50 with confidence limits	Greater than 5000 mg/kg bw
Number of deaths at each dose level	1 death on day 7 1 death on day 13
Remarks for Results	The rabbit that died on day 7 appeared emaciated, lethargic and ptotic with discharge from nose and eyes 1 day prior to

	death. At necropsy, this animal had dried fecal material in anogenital region, exudate in the nose and mouth, small spleen, mottled kidney and redness in portions of the colon. At necropsy of the other animals, 1 rabbit had blotchy liver, 2 had mottled kidneys, and 1 had yellowish nodules on the liver. With respect to skin irritation, redness was slight in 5 rabbits, moderate in 4 rabbits and severe in 1 rabbit. Edema was slight in 2 rabbits and moderate in 8 rabbits.
Conclusion remarks	The dermal LD50 of HMPCC in rabbits was reported to be greater than 5,000 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Opdyke D.L. (1977) Acute Oral Toxicity in Rats. Dermal Toxicity in Rabbits, Lyral. Unpublished report to RIFM.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Purity greater than 88%; 0.02% acrolein; less than 10% myrac, less than 2.5% myrcenol; mol wt = 210.3
Test Type	Acute dermal LD50
GLP	No
Year	1987
Species/strain	Rabbit
Sex	Male and Female
# of animals per sex per dose	5
Route of Administration	Dermal
Remarks for Test Conditions	Groups of male and female rabbits were administered a single dose of HMPCC, 4.0, 8.0 or 16.0 ml/kg bw by dermal application and observed for 14 days.
Value LD50 or LC50 with confidence limits	LD50 for males = 11.3 ml/kg bw (95% CL = 4.5-28.5) LD50 for females = 13.5 ml/kg bw (95% CL = 5.4-33.6)
Number of deaths at each dose level	Deaths occurred between 1 and 3 days following treatment. 4.0 ml/kg bw - 0/5 males; 0/5 females 8.0 ml/kg bw - 2/5 males; 1/5 females 16 ml/kg bw - 3/5 males; 3/5 females
Remarks for Results	Signs exhibited included sluggishness, unsteady gait, nasal discharge, salivation and prostration. Dermal effect included erythema, edema, ecchymosis, necrosis, fissuring, crusty

	texture, desquamation, scabs, alopecia and ulceration. Gross pathology showed subcutaneous edema, mottled and red lungs and tracheas with red patches. Survivors showed recovery within 7-14 days.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Myers R.C., Slesinski R.S., Frank F.R. (1987) Lyral (crude) [4-(4-hydroxy-4-methyl pentyl)-3-cyclohexene-1-carboxaldehyde]. Acute Toxicity and Primary Irritancy Studies. Bushy Run Research Center, Export, PA. Report No. 49-180 dated February 6, 1987.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data is for structurally related substance 7-hydroxycitronellal
Test Type	Acute dermal toxicity
GLP	No
Year	1973
Species/strain	Rabbit
Sex	Not reported
# of animals per sex per dose	2
Route of Administration	Dermal
Remarks for Test Conditions	Rabbits were topically administered 2000 mg/kg of 7-hydroxycitronellal and observed for 14 days.
Value LD50 or LC50 with confidence limits	Greater than 2000 mg/kg.
Number of deaths at each dose level	No deaths.
Remarks for Results	No symptomatology reported; however there was insufficient material for a complete determination.
Conclusion remarks	The dermal LD50 of 7-hydroxycitronellal in rabbits was reported to be greater than 2,000 mg/kg bw.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Opdyke D.L. (1973) Acute oral toxicity in rats. Dermal toxicity in rabbits. Unpublished report to RIFM.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data is for structurally related substance 4,4-dimethyl-3-cyclohexenecarboxaldehyde
Test Type	Acute vapor inhalation toxicity test
GLP	No
Year	1986
Species/strain	Rat/Sprague-Dawley
Sex	Male and Female
# of animals per sex per dose	5
Route of Administration	Inhalation
Remarks for Test Conditions	Groups of rats (5/sex) were exposed in a dynamic system to 459, 521, or 558 ppm 4,4-dimethyl-3-cyclohexenecarboxal vapor for 4 hours and observed for 14 days. Similarly groups of rats (5/sex) were exposed to 365 or 402 ppm 4,4-dimethyl-3-cyclohexenecarboxaldehyde vapor for 1 hour in a static system and observed for 14 days. In the dynamic system, compressed air was passed through a bottle containing crude 4,4-dimethyl-3-cyclohexenecarboxaldehyde and the vapor entered the inhalation chamber either undiluted or diluted to the target concentration with filtered air. For the 521 ppm exposure group, a fresh test sample was introduced after 2 hours. For the static exposure groups, the test substance was left in a sealed 120 L chamber for 18-19 hours prior to introducing the rats. For the 365 ppm exposure, the test material was sparged with N2 gas for 2 hours prior to enclosing in the chamber.
Number of deaths at each dose level	Deaths occurred on days 1 or 2 post-exposure. Dynamic system: 521 ppm: 1/5 males; 3/5 females 459 and 558 ppm: 0/5 males; 0/5 females Static system: 365 and 402 ppm: 0/5 males; 0/5 females
Remarks for Results	Dynamic system At 558 ppm, acrolein vapor of 42 ppm was detected. After 20 min, no acrolein vapor was detected (DL = 10 ppm). Signs exhibited included lacrimation, perioral wetness and respiratory difficulties on day of exposure. No clinical signs or macroscopic lesions were reported post exposure. Static system For 365 and 402 ppm, acrolein vapor was 2.0 and 8.4 ppm, respectively. Signs exhibited included lacrimation and periocular wetness. No clinical signs or macroscopic lesions

	were reported post exposure.
Conclusion remarks	The authors considered the deaths reported at 558 ppm to be related to the initial acrolein exposure of 42 ppm since the 1-hour rat LC5 of acrolein is 26 ppm. Therefore, no mortalities were attributed to 4,4-dimethyl-3-cyclohexenecarboxaldehyde exposure.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Union Carbide (1987) Aldehyde AA (crude). Acute vapor inhalation toxicity test. Bushy Run Research Center. Project No. 50-54 dated April 27, 1987.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Purity greater than 88%; 0.02% acrolein; less than 10% myrac, less than 2.5% myrcenol; mol wt = 210.3
Test Type	Static 6-hour inhalation toxicity test
GLP	No
Year	1987
Species/strain	Rat
Sex	Male and Female
# of animals per sex per dose	5
Route of Administration	Inhalation
Remarks for Test Conditions	Groups of male and female rats were exposed to a statically generated substantially saturated HMPCC vapor for 6 hours and observed for 14 days.
Number of deaths at each dose level	No deaths occurred.
Remarks for Results	No signs of toxicity and no remarkable gross pathology.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Myers R.C., Slesinski R.S., Frank F.R. (1987) Lyral (crude) [4-(4-hydroxy-4-methyl pentyl)-3-cyclohexene-1-carboxaldehyde]. Acute Toxicity and Primary Irritancy Studies. Bushy Run Research Center, Export, PA. Report No. 49-180 dated February 6, 1987.

4.2 Genetic Toxicity

4.2.1 *In vitro* Genotoxicity

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Substance	Data is for structurally related substance 2,4-dimethyl-3-cyclohexene-1-carboxaldehyde
Method/guideline	Plate incorporation method
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	Yes
Year	1995
Species/Strain	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537, and TA1538
Metabolic Activation	S9 from Aroclor 1254-induced rat liver
Doses/Concentration	0.03, 0.10, 0.30, 1.0, and 3.0 mg/plate
Remarks for Test Conditions	A preliminary cytotoxicity assay was conducted to determine appropriate concentrations for the mutagenicity study. 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde was tested at concentrations of 0.03, 0.10, 0.30, 1.0, or 3.0 mg/plate. Acetone was the solvent used. The positive controls used were 4-nitro-o-phenylenediamine, sodium azide, 2-aminoanthracene, and 9-aminoacridine. Treated cultures (in triplicate) were incubated in the presence or absence of S9 at 37 deg C for 48-72 hours. Colonies were counted either manually or with an Artek Model No. 880 Colony Counter. The test substance was considered positive for mutagenicity if it "consistently produced a dose-related increase in the mean reversion frequency of at least one bacterial strain as compared to the vehicle control for that strain. At least one of those doses must have produced a mean reversion frequency at least twice that of the vehicle control." The test substance was also considered a bacterial mutagen if a "reproducible increase in the mean number of revertant colonies at a single dose level of at least 2-fold compared to the vehicle control" was noted.
Results	Mean plate counts without S9 for solvent control, 0.03, 0.10, 0.30, 1.0 and 3.0 mg/plate: TA98: 24, 22, 22, 23, 19, and toxic TA100: 98, 89, 99, 91, 86, and toxic TA1535: 7, 10, 9, 9, 4, and toxic

	TA1537: 7, 6, 6, 5, 5, and toxic TA1538: 11, 13, 14, 16, 14, and toxic Mean plate counts with S9 for solvent control, 0.03, 0.10, 0.30, 1.0 and 3.0 mg/plate: TA98: 34, 23, 27, 31, 27, and 17 TA100: 106, 99, 114, 112, 96, and 82 TA1535: 11, 11, 9, 8, 9, and 8 TA1537: 6, 5, 6, 3, 6, and 3 TA1538: 20, 19, 15, 17, 3 (toxic), and 10 Similar results were reported in a repeat experiment. All 5 strains showed appropriate responses to the positive controls.
Cytotoxic concentration	1.0 mg/plate
Genotoxic Effects	None
Conclusion Remarks	2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde was not a bacterial mutagen in this assay.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Vergnes J.S., Morabit, E.R. [1995] Aldehyde AA (Crude): Mutagenic Potential in the Salmonella/Microsome (Ames) Assay. 2,4-Dimethyl-3-cyclohexene-1-carboxaldehyde. Bushy Run Research Center. No. 94U1472. March 3, 1995.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Plate incorporation method (Maron and Ames, 1983)
Test Type	Ames reverse mutation
System of Testing	Bacterial
GLP	Yes
Year	1999
Species/Strain	*Salmonella typhimurium* strains TA98, TA100, TA1535, TA1537; *Escherichia coli* strain WP2 uvrA
Metabolic Activation	S9 from Aroclor 1254-induced rat liver
Doses/Concentration	Vehicle, 75, 200, 600, 1,800, and 5,000 ug/plate

Remarks for Test Conditions	Dimethyl sulfoxide was used as the test vehicle. A preliminary toxicity assay was conducted using concentrations up to 5,000 ug/plate to determine appropriate test concentrations. Lyral was tested both in the presence and absence of S9. Plates were plated in triplicate and incubated for 48 to 72 hours at 37 deg C. Any plates not counted immediately were stored at 2-8 deg C. Colony counting was conducted either entirely by automated colony counter or entirely manually. Any plates with sufficient precipitate to interfere with the automatic counter were counted manually. Positive controls used were 2-aminoanthracene, 2-nitrofluorene, sodium azide, 9-aminoacridine and methyl methansulfonate. To be considered a positive finding, the increase in mean revertants at the peak of the dose response must have been equal to 3 times (for TA1535 and TA1537) or 2 times (for TA98, TA100 and WP2 uvrA) the mean vehicle control value. The test substance was considered positive if it caused a dose-related increase in the mean revertants/plate of at least one tester strain with a minimum of 2 increasing concentrations.
Results	No precipitate was observed. Toxicity was reported in strains TA98 and TA1537 at 5,000 ug/plate. No positive responses were reported; however, a non-dose-related increase was reported in TA98 (1.8-fold increase) and TA100 (1.5-fold increase) in the absence of S9. Lyral was retested in TA98 and TA100 without S9 resulting in a non-dose-related increase in TA98 (2.4-fold) and no effect in TA100. The average revertants/plate in the absence of S9 are as follows for vehicle, 75, 200, 600, 1,800, 5,000 ug/plate and positive control, respectively: TA98: 14, 10, 16, 21, 25, 10 and 486 TA100: 135, 148, 159, 174, 169, 202, and 582 TA1535: 7, 7, 8, 7, 11, 9, and 459 TA1537: 8, 10, 8, 10, 10, 3, and 617 WP2 uvrA: 17, 19, 17, 12, 13, 13, and 173 The average revertants/plate in the presence of S9 are as follows for vehicle, 75, 200, 600, 1,800, 5,000 ug/plate and positive control, respectively: TA98:20, 22, 20, 22, 27, 25, and 851 TA100: 173, 180, 190, 177, 177, 203, and 706 TA1535: 9, 9, 12, 13, 13, 8, and 72 TA1537: 10, 11, 9, 12, 11, 9, and 71 The average number of revertants/plate in the repeat assay for strains TA98 and TA100 without S9 were as follows for vehicle, 75, 200, 600, 1,800, 2,500, 5,000 ug/plate and positive control: TA98: 10, 14, 14, 24, 18, 16, 8, and 599 TA100: 166, 177, 188, 197, 198, 184, 177, and 574
Cytotoxic concentration	5,000 ug/plate

Genotoxic Effects	No positive findings.
Remarks for Results	The increase in revertant count reported in TA98 without metabolic activation was within the normal historical vehicle control range and not considered to be biologically relevant by the testing laboratory.
Conclusion Remarks	The test substance was considered to test negative in the bacterial reverse mutation assay.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Wagner V.O., Klug, M.L. [1999] Bacterial Reverse Mutation Assay. 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde; CAS Registry #31906-04-4. BioReliance, Rockville, MD. Study No. AA10BX.502.BTL, October 4, 1999. Maron, D.M., Ames, B.N. [1983] Revised methods for the Salmonella mutagenicity test. Mutat Res 38:3-32.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Chromosomal aberration (Evans, 1976)
Test Type	Clastogenic assay
System of Testing	Mammalian
GLP	Yes
Year	2000
Species/Strain	Chinese hamster ovary cell
Metabolic Activation	S9 from Aroclor 1254-induced rat liver
Doses/Concentration	4-hour without S9: vehicle, 200, 400, and 600 ug/ml 4-hour with S9: vehicle, 200, 800, and 900 ug/ml 20-hour without S9: vehicle, 100, 200, and 400 ug/ml
Remarks for Test Conditions	A preliminary toxicity assay at concentrations up to 2,100 ug/ml was conducted to determine appropriate test concentrations. Dimethyl sulfoxide was used as the test vehicle. Chinese hamster ovary cells were treated for 4 or 20 hours in the absence of S9 or they were treated for 4 hours in the presence of S9. At 20 hours, all cells were harvested. 50% cell growth inhibition compared to vehicle control was considered substantial toxicity. Cells were seeded in flasks and incubated at 37 deg C for 16-24 hours. Duplicate cultures were exposed to lyral, positive controls (Mitomycin C and cyclophosphamide), and DMSO vehicle. In cultures without S9, cells were exposed to the test substance for 4 hours after which the treatment medium was removed, the cells were washed with CMF-PBS,

and then re-incubated in complete medium. Cultures without S9 also were exposed continuously to the test substance for 20 hours. Two hours prior to harvest (at 20 hours after treatment initialization), 0.1 ug Colcemid/ml was added to the flasks. Cultures incubated in the presence of S9 were treated exactly the same as those exposed without S9 for 4 hours. Cells were collected and stored overnight in fixative at 2-8 deg C. Slides were prepared using Giemsa staining and metaphase cells with 20+/-2 centromeres were examined under oil immersion and evaluated. If a positive result was obtained in the non-activated 4-hour group, then the non-activated 20-hour group was not evaluated for chromosome aberrations. An attempt was made to examine a minimum of 100 metaphase spreads per duplicate flask and score for chromatid-type and chromosome-type aberrations.

For both S9 and non-S9 systems, a concurrent toxicity test was performed by removing an aliquot of cell suspension post cell harvest. Cells in the aliquot were counted using a Coulter counter, precipitate was noted, and cell viability was determined using trypan blue dye. Cell growth inhibition relative to DMSO vehicle were determined using cell counts and percent viability. The test substance was considered positive if the percentage of cells with aberrations showed a dose-related increase with one or more concentrations showing statistical significance ($p<=0.05$). The number and types of aberrations found, the percentage of structurally and numerically damaged cells (percent aberrant cells) in the total population of cells examined, and the mean aberrations per cell was calculated and reported for each group.

Results

Percent cells with aberrations (structural) **=p<=0.01:

4-hour without S9 for vehicle, 200, 400, 600 ug/ml and MMC: 0.0, 0.0, 7.0**, 0.5, and 12.0**

4-hour with S9 for vehicle, 200, 800, 900 ug/ml, and CP: 0.5, 8.5**, 11.0**, 24,0**, and 22.5**

20-hour without S9 for vehicle, 100, 200, 400 ug/ml, and MMC: 0.0, 0.5, 1.5, 3.5**, and 13.0**

Percent cells with aberrations (numerical) :

4-hour without S9 for vehicle, 200, 400, 600 ug/ml and MMC: 1.5, 0.5, 2.0, 3.5, and 3.5

4-hour with S9 for vehicle, 200, 800, 900 ug/ml, and CP: 3.5, 4.5, 4.0, 5.5, and 4.5

20-hour without S9 for vehicle, 100, 200, 400 ug/ml, and MMC: 2.0, 2.5, 1.0, 2.0, and 2.5

Cytotoxic concentration

Greater than or equal to 630 ug/ml at 4 hours without S9; 2,100 ug/ml at 4 hours with S9; 210 and 2,100 ug/ml at 20 hours without S9

Genotoxic Effects

Lyral was considered to induce structural chromosomal aberrations in the presence of S9, but not in the absence of S9. Lyral did not induce numerical chromosome aberrations in the

	presence or absence of S9.
Appropriate statistical evaluations?	Yes. Fisher's exact test and Cochran-Armitage test.
Remarks for Results	Since the statistically significant increase in the percentage of structurally aberrant cells at 400 ug/ml in the non-activated 4-hour group (7%) was only 1% outside of the historical solvent control range (0-6%) and in the non-activated 20-hour group (3.5%) was within the historical control range, these increases were not considered by the study authors to be biologically significant.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Guideline study.
References	Gudi R. and Schadly, E.H. [2000] In Vitro Mammalian chromosome Aberration Test. 3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde; CAS Registry #31906-04-4. BioReliance, Rockville, MD. Study No. AA10BX.331.BTL, May 19, 2000. Evans, H.J. [1976] Cytological methods for detecting chemical mutagens. In: Hollaender, A. (Ed.) Chemical Mutagens, Principles and Methods for their Detection. Volume 4. Plenum Press, New York.

4.2.2 *In vivo* Genotoxicity

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data is for structurally related substance 7-hydroxycitronellal
Method/guideline	Sex linked recessive lethal mutation assay (Wuergler *et al.*, 1977)
Test Type	Lethal mutation test
GLP	No
Year	1982
Species/Strain	*Drosophila melanogaster*
Sex	Not reported
Route of Administration	Oral-Diet

Doses/Concentration	37 mM
Remarks for Test Conditions	Flies were exposed to the test compound prepared in a 5% saccharose solution and 2% ethanol and 2% Tween 80 for compounds with poor water solubility. Further details of the methodology were not reported.
Genotoxic effects	None
Appropriate statistical evaluations	Yes. Statistical significance determined by methods of Kastenbaum and Bowman (1970).
Remarks for Results	Number (%) of sex-linked recessive lethals/chromosomes tested for Brood I, II, and III, respectively: 4/1,222; 1/687; and 0/95.
Conclusion Remarks	Hydroxycitronellal did not increase the number of sex-linked recessive lethal mutations as compared to controls.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Wild D., King M.-T., Gocke E. and Eckhardt K. (1983). Study of artificial flavouring substances for mutagenicity in the *Salmonella*/microsome, Basc and micronucleus tests. Fd Chem Toxicol 21(6):707-719. Kastenbaum, M.A. and Bowman K.O. (1970). Tables for determining the statistical significance of mutation frequencies. Mutat Res 9:527. Wuergler F.E., Sobels F.H., and Vogel E. (1977). Drosophila as assay system for detecting genetic changes. In Handbook of Mutagenicity Test Procedures. Kilbey, B.J., Legator, M., Nichols, W. and Ramel, C. (eds.) Elsevier, Amsterdam, p. 335.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data is for structurally related substance hydroxycitronellol
Method/guideline	Sex linked recessive lethal mutation assay (Wuergler *et al.*, 1977)
Test Type	Lethal mutation test
GLP	No
Year	1982
Species/Strain	*Drosophila melanogaster*
Sex	Not reported
Route of Administration	Oral-Diet

Doses/Concentration	10 mM
Remarks for Test Conditions	Flies were exposed to the test compound prepared in a 5% saccharose solution and 2% ethanol and 2% Tween 80 for compounds with poor water solubility. Further details of the methodology were not reported.
Genotoxic effects	None
Appropriate statistical evaluations	Yes. Statistical significance determined by methods of Kastenbaum and Bowman (1970).
Remarks for Results	Number (%) of sex-linked recessive lethals/chromosomes tested for Brood I, II, and III, respectively: 3/1,227; 1/1,208; and 0/1,211.
Conclusion Remarks	Hydroxycitronellol did not increase the number of sex-linked recessive lethal mutations as compared to controls.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Wild D., King M.-T., Gocke E. and Eckhardt K. (1983). Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, Basc and micronucleus tests. Fd Chem Toxicol 21(6):707-719. Kastenbaum M.A. and Bowman K.O. (1970). Tables for determining the statistical significance of mutation frequencies. Mutat Res 9:527. Wuergler F.E., Sobels F.H., and Vogel E. (1977). Drosophila as assay system for detecting genetic changes. In Handbook of Mutagenicity Test Procedures. Kilbey, B.J., Legator, M., Nichols W. and Ramel C. (eds.) Elsevier, Amsterdam, p. 335.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Method/guideline	Micronucleus test (Heddle, 1973; Hayashi *et al.*, 1994; Mavournin *et al.*, 1990)
Test Type	Clastogenic assay
GLP	Yes
Year	2000
Species/Strain	Mouse/ICR
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/Concentration	Corn oil vehicle, 225, 450, and 900 mg/kg bw

Exposure Period	Single dose
Remarks for Test Conditions	A preliminary pilot study and toxicity assay were conducted to determine appropriate dosages for the micronucleus assay. Groups of 5 male and 5 female mice were intraperitoneally injected with corn oil vehicle, 225, 450 or 900 mg lyral/kg bw. Ten additional mice per sex were given the high-dose and 5/sex were designated as replacement animals in the event of high mortality and 5/sex were used for bone marrow collection at 48 hours. Similarly, an additional 5 mice/sex were given corn oil vehicle and used for bone marrow collection at 48 hours. Positive control mice were administered 50 mg cyclophosphamide/kg bw. At 24 hours, and, in the case of vehicle and high-dose mice, at 48 hours, mice were killed, femurs were exposed and bone marrow was removed. Two slides per mouse were prepared and were fixed in methanol, stained with May-Gruenwald-Giemsa and permanently mounted. Two thousand polychromatic erythrocytes were scored for the presence of micronuclei using oil immersion. The number of micronucleated normochromatic erythrocytes was counted and the proportion of polychromatic erythrocytes to total erythrocytes was determined. A positive response was concluded if a dose-related increase in micronucleated polychromatic erythrocytes was reported with one or more doses showing a statistically significant increase relative to the vehicle control.
Effect on mitotic index or PCE/NCE ratio by dose level and sex	Piloerection and lethargy were noted in all mice treated with lyral. At 900 mg/kg bw, one female mouse died and was replaced with one from the designated replacements. In addition, mice of both sexes showed irregular breathing at the highest dose. PCE/Total Erythrocytes (mean) at 24 hours for corn oil vehicle, 225, 450, 900 mg/kg bw, and CP: Males: 0.544, 0.486, 0.429, 0.406, and 0.339 Females: 0.515, 0.395, 0.403, 0.415, and 0.350 PCE/Total Erythrocytes (mean) at 48 hours for corn oil vehicle, and 900 mg/kg bw: Males: 0.539 and 0.372 Females: 0.526 and 0.385
Genotoxic effects	None
Appropriate statistical evaluations	Yes. Kastenbaum-Bowman
Conclusion Remarks	Lyral did not induce micronucleated polychromatic erythrocytes in mouse bone marrow.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Gudi R. and Krsmanovic, L. (2000) Mammalian Erythrocyte Micronucleus Test. 3 and 4-(4-Hydroxy-4-methylpentyl)-3-

cyclohexene-1-carboxaldehyde; CAS Registry #31906-04-4. BioReliance, Rockville, MD. Study No. AA10BX.123.BTL, June 30, 2000.

Heddle, J.A. [1973] A rapid in vivo test for chromosomal damage. Mutat Res 18:187-190.

Hayashi, M., Tice, R.R., MacGregor, J.T., Anderson, D., Blakey, D.H., Dirsch-Volders, M., Oleson, Jr., F.G., Pacchierotti, F., Romagna, F., Shimada, H., Sutou, S., Vannier, B. [1994] In vivo rodent erythrocyte micronucleus assay. Mutat Res 312:293-304.

Mavournin, K.H., Blakey, D.H., Cimino, M.C., Salamone, M.F., Heddle, J.A. [1990] The in vivo micronucleus assay in mammalian bone marrow and peripheral blood. A report of the US Environmental Protection Agency Gene-Tox Program. Mutat Res 239:29-80.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data is for structurally related substance hydroxycitronellol
Method/guideline	Micronucleus test
Test Type	Clastogenic assay
GLP	No
Year	1982
Species/Strain	Mouse/NMRI
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/Concentration	0, 516, 860, and 1,204 mg/kg
Exposure Period	Single dose
Remarks for Test Conditions	Groups of 10- to 14-week-old NMRI mice were given a single intraperitoneal injection of 0, 516, 860, or 1,204 mg/kg bw of hydroxycitronellol. At 30 hours, the mice were killed and bone marrow smears were prepared using the staining method of Schmid (1976).
Effect on mitotic index or PCE/NCE ratio by dose level and sex	The mean number of micronucleated PE/1000 PE at 0, 516, 860, and 1,204 mg/kg bw was 2.0, 2.2, 2.2, and 2.6 respectively.
Genotoxic effects	None
NOEL (C)/ LOEL (C)	1,204 mg/kg bw
Appropriate statistical evaluations	Yes. Statistical significance determined by methods of Kastenbaum and Bowman (1970).

evaluations	Kastenbaum and Bowman (1970).
Conclusion Remarks	Hydroxycitronellol did not induce micronuclei in this assay.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Wild D. King, M.-T., Gocke, E. and Eckhardt K. (1983) Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, Basc and micronucleus tests. Fd Chem Toxicol 21(6):707-719. Kastenbaum, M.A. and Bowman, K.O. (1970). Tables for determining the statistical significance of mutation frequencies. Mutat Res 9:527. Schmid, W. [1976] The micronucleus test for cytogenetic analysis. In: Hollaender, A. (ed) Chemical Mutagens, Vol. 4, p. 31. Plenum Press, NY.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data is for structurally related substance 7-hydroxycitronellal
Method/guideline	Micronucleus test
Test Type	Clastogenic assay
GLP	No
Year	1982
Species/Strain	Mouse/NMRI
Sex	Male and Female
Route of Administration	Intraperitoneal
Doses/Concentration	0, 345, 603, and 861 mg/kg
Exposure Period	Single dose
Remarks for Test Conditions	Groups of 10- to 14-week-old NMRI mice were given a single intraperitoneal injection of 0, 345, 603, and 861 mg hydroxycitronellal/kg bw. At 30 hours, the mice were killed and bone marrow smears were prepared using the staining method of Schmid (1976).
Effect on mitotic index or PCE/NCE ratio by dose level and sex	The mean number of micronucleated PE/1000 PE at 0, 345, 603, and 861 mg/kg bw was 1.5, 1.5, 1.0, and 2.0, respectively.
Genotoxic effects	None
NOEL (C)/ LOEL (C)	861 mg/kg bw

Appropriate statistical evaluations	Yes. Statistical significance determined by methods of Kastenbaum and Bowman (1970).
Conclusion Remarks	Hydroxycitronellal did not induce micronuclei in this assay.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	Wild D., King, M.-T., Gocke E. and Eckhardt K. (1983). Study of artificial flavouring substances for mutagenicity in the Salmonella/microsome, Basc and micronucleus tests. Fd Chem Toxicol 21(6):707-719. Kastenbaum, M.A. and Bowman, K.O. (1970). Tables for determining the statistical significance of mutation frequencies. Mutat Res 9:527. Schmid, W. [1976] The micronucleus test for cytogenetic analysis. In: Hollaender, A. (ed) Chemical Mutagens, Vol. 4, p. 31. Plenum Press, NY.

4.3 Repeated Dose Toxicity

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data for structurally related substance, 4-isopropenyl-1-cyclohexencarbinol
Method/guideline	90-Day oral study
GLP	Ambiguous
Year	1996
Species/strain	Rat/Fischer F344
Sex	Male and Female
Route of Administration	Oral-Intragastric
Doses/concentration Levels	40, 120, or 400 mg/kg bw
Exposure Period	90 days
Frequency of Treatment	Daily
Remarks for Test Conditions	Groups of male and female rats (10/sex/group) were administered to 40,120, or 400 mg/kg bw of 4-isopropenyl-1-

	cyclohexenecarbinol by gastric intubation in soybean oil once daily for 90 days. Animals were observed daily for clinical signs and body weights were measured on weekly. Hematological examination and clinical chemistry determinations were monitored at conclusion of the study. At necropsy, organ weights were measured and histopathological evaluation was performed.
NOAEL(NOEL)	120 mg/kg per day
LOAEL(LOEL)	400 mg/kg per day
Toxic Response/effects by Dose Level	No mortalities were recorded during the study. A significant decrease in body weight was reported in the high-dose group of males. Although absolute kidney, liver and lungs weights were increased in high-dose females, there was no evidence of histopathology in any of these organs.
Remarks for Results	The authors reported that despite a significant weight loss in high-dose males, the NOEL is greater than 400 mg/kg bw per day. Based on the increased organ weights in females, the NOAEL was reported to be less than 400 mg/kg bw per day.
Conclusion Remarks	The NOAEL for 4-isopropenyl-1-cyclohexenecarbinol in Fischer F344 rats is 120 mg/kg bw per day
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Basic data given: comparable to guidelines/standards.
References	National Cancer Institute (1996) Clinical Development Plan: l-Perillyl alcohol. Journal of Cellular Biochemistry. 265, 137-148.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data for structurally related substance 7- hydroxycitronellal
Method/guideline	2-year feeding study
GLP	No
Year	1967
Species/strain	Rat
Sex	Male and Female
Route of Administration	Oral-Diet
Doses/concentration Levels	0.1 and 0.5% in the diet (approximately 50 and 250 mg/kg bw/day)
Exposure Period	2 years
Frequency of Treatment	Daily

Control Group	Basal diet
Remarks for Test Conditions	Groups of rats of both sexes were fed a diet containing hydroxycitronellal at 0.1 (10 rats/sex) or 0.5% (20 rats/sex) for a period of 2 years. At the end of the study, rats were necropsied and microscopic examinations were performed on the liver, heart, pancreas, adrenals, spleen, brain, and gross lesions.
NOAEL(NOEL)	0.5% (250 mg/kg bw/day)
Toxic Response/effects by Dose Level	No adverse effects were reported. The number of rats at the beginning of the study, after 1 year, after 1.5 years and at the end of the study were as follows: 0.1%: 20, 10, 7, and 5 0.5%: 60, 50, 48, and 31
Remarks for Results	This study was reported in German.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Bär F. and Griepentrog F. (1967) Die Situation in der gesundheitlichen Beurteilung der Aromatisierungsmittel für Lebensmittel. Med Emahr 8:244.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data for structurally related substance 4,4-dimethyl-3-cyclohexenecarboxaldehyde
Method/guideline	9-Day vapor inhalation toxicity study
GLP	Yes
Year	1992
Species/strain	Rat/CD
Sex	Male and Female
Route of Administration	Inhalation
Doses/concentration Levels	0, 50, 125 and 250 ppm
Exposure Period	6 hours
Frequency of Treatment	Daily for 4 consecutive days, 2 days off, then another 5 consecutive days
Control Group	Yes
Remarks for Test Conditions	Groups of 10 rats/sex were exposed to 0, 50, 125 or 250 ppm aldehyde AA vapor, 6 hours/day for 9 exposures. An additional 5 rats/sex were assigned to the control and high concentration

	groups for inclusion in a 4-week recovery period. The vapor was generated using a reservoir system in which compressed air was blown across the head space of liquid test substance. The test chambers were analyzed for 4,4-dimethyl-3-cyclohexenecarboxaldehyde content 4 times during the 6-hour exposure period. The chamber O2 content was 20.8%. Animals were observed daily for clinical signs, body weights were measured on days 2, 4, 7, 8, and termination, hematology and clinical chemistry determinations were conducted, and necropsies were performed. *alpha*-2 micro-globulin immunohistochemical evaluations were conducted on males from the day 12 terminations.
LOAEL(LOEL)	50 ppm
Toxic Response/effects by Dose Level	50 ppm: decreased bw gain on days 1-2 in males; increased water consumption for days 9-10 in males, increased water consumption for days 1-9 and 9-11 in females; increased serum urea nitrogen values in males; increased incidence of higher values for bilirubin, urobilinogen and amorphous phosphates in males on day 11
	125 ppm: swollen periocular tissue; decreased bw gain on days 1-2; increased water consumption for days 9-10 in males, increased water consumption for days 1-9 and 9-11 in females; increased serum urea nitrogen values in males; increased urine osmolalities in males on day 11; increased incidence of higher values for bilirubin, urobilinogen and amorphous phosphates in males on day 11; increased relative liver weights in males; exposure related increase in renal tubular immunohistochemical staining for *alpha*-2micro-globulin in males
	250 ppm: swollen periocular tissue, periocular encrustation, alopecia; decreased bw gain on days 1-2 in males, increased bw gain on days 2-4 in males, decreased bw gain on days 1-2 in females; increased water consumption for days 1-10 in males, increased water consumption for days 1-9 and 9-11 in females; corneal lesions; after the recovery period females had decreased total erythrocytes, hemoglobin, hematocrit, and mean corpuscular hemoglobin concentration values; after the recovery period females also showed increased segmented neutrophils and decreased monocytes; increased serum urea nitrogen values; increased urine osmolalities in males on day 11; increased incidence of higher values for bilirubin, urobilinogen and amorphous phosphates in males on day 11; increased incidence of bilirubin and urobilinogen in the urine of females on day 12; after recovery period females showed slightly increased protein in the urine; increased relative liver and kidney weights in males; exposure related increase in renal tubular immunohistochemical staining for *alpha*-2micro-globulin in males
Appropriate statistical evaluations?	Yes, ANOVA, t-tests, Kruskal-Wallis test, Mann-Whitney U-test, Fisher's exact test.

Conclusion Remarks	4,4-dimethyl-3-cyclohexenecarboxaldehyde appears to be a ocular and respiratory irritant at vapor concentrations of 125 ppm and higher.
Data Qualities Reliabilities	Reliability code 1. Reliable without restriction.
Remarks for Data Reliability	Code 1. Comparable to guideline study.
References	Norris J.C. and Kintigh, W.J. (1994) (Crude) Aldehyde AA: Nine-day vapor inhalation toxicity study in rats. Bushy Run Research Center, Export, PA. Project ID No. 92U1012. Dated December 16, 1994.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data for structurally related substance 7-hydroxycitronellal
Method/guideline	Subchronic inhalation toxicity study
GLP	Ambiguous
Year	1999
Species/strain	Rat/CD
Sex	Female
Route of Administration	Inhalation
Doses/concentration Levels	Mixture:50 mg/m3 Hydroxycitronellal: 211 ug/m3
Exposure Period	13 weeks
Frequency of Treatment	4 hour/day, 5 days/week
Remarks for Test Conditions	Twelve animals per test group were subjected to a whole body inhalation exposure experiment. Aerodynamic mean diameter of particle size was 0.5 um. Animals were sacrificed 1 to 2 days following exposure. Hematological examination at week 13 involved measurement of white blood cell count, mean corpuscular volume, hemoglobin concentration, and hematocrit. Clinical chemistry examinations were performed at weeks 6 or 7 and week 13. At necropsy, gross pathological examination was performed on 24 organs and tissues including the uterus, testes and ovaries. Histopathological examination was performed on the trachea, lungs, adrenals, brain, esophagus, heart, kidneys, liver pancreas, spleen, sternum, testes, uterus and bone marrow taken from the femur. Data from all studies were subjected to a Student's t-test. The significance level was chosen to be P less than 0.05.
NOAEL(NOEL)	211 ug/m3

Toxic Response/effects by Dose Level	No toxicologically significant effects on animal survival, behavior, body weights or weight gains, organ weights, or in hematology, clinical chemistry, or urinalysis parameters were reported and no gross pathological or histopathological findings were observed.
Appropriate statistical evaluations?	Yes. Student's t-test.
Remarks for Results	There were no adverse effects reported after female rats were exposed to an aerosol mixture (50 mg/m3) containing 211 ug/m3 of 7-hydroxycitronellal for 4 hours daily, 5 days per week for 13 weeks.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Fukayama M.Y., Easterday, O.D., Serafino, P.A., Renskers, K.J., North-Root, H., Schrankel, K.R. (1999) Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters. Toxicol Lett 111:175-187.

Substance Name	3 and 4-(4-Hydroxy-4-methylpentyl)-3-cyclohexene-1-carboxaldehyde
CAS No.	31906-04-4
Remarks for Test Substance	Data for structurally related substance 7-hydroxycitronellal
Method/guideline	Subchronic inhalation toxicity study
GLP	Ambiguous
Year	1999
Species/strain	Hamster/Syrian golden
Sex	Female
Route of Administration	Inhalation
Doses/concentration Levels	Mixture: 50 mg/m3 Hydroxycitronellal: 211 ug/m3
Exposure Period	13 weeks
Frequency of Treatment	4 hour/day, 5 days/week
Remarks for Test Conditions	Twelve animals per test group were subjected to a whole body inhalation exposure experiment. Aerodynamic mean diameter of particle size was 0.5 um. Animals were sacrificed 1 to 2 days following exposure. Hematological examination at week 13 involved measurement of white blood cell count, mean corpuscular volume, hemoglobin concentration, and hematocrit. Clinical chemistry examinations were performed at weeks 6 or 7 and week 13. At necropsy, gross pathological examination was performed on 24 organs and tissues including the uterus,

	testes and ovaries. Histopathological examination was performed on the trachea, lungs, adrenals, brain, esophagus, heart, kidneys, liver pancreas, spleen, sternum, testes, uterus and bone marrow taken from the femur. Data from all studies were subjected to a Student's t-test. The significance level was chosen to be P less than 0.05.
NOAEL(NOEL)	211 ug/m3
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Appropriate statistical evaluations?	Yes. Student's t-test.
Remarks for Results	There were no adverse effects reported after female rats were exposed to an aerosol mixture (50 mg/m3) containing 211 ug/m3 of 7-hydroxycitronellal for 4 hours daily, 5 days per week for 13 weeks.
Data Qualities Reliabilities	Reliability code 2. Reliable with restriction.
Remarks for Data Reliability	Code 2. Comparable to guideline study with acceptable restrictions.
References	Fukayama, M.Y., Easterday, O.D., Serafino, P.A., Renskers, K.J., North-Root, H., Schrankel, K.R. (1999) Subchronic inhalation studies of complex fragrance mixtures in rats and hamsters. Toxicol Lett 111:175-187.